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Soil Biology & Biochemistry 35 (2003) 1675–1682

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Rhizobial and bradyrhizobial symbionts of mesquite from the Sonoran Desert: salt tolerance, facultative halophily and nitrate respiration

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Received 20 December 2002; received in revised form 15 July 2003; accepted 4 August 2003

Abstract

Rhizobial symbionts were isolated from the surface (0–0.5 M) and phreatic (3.9–5.0 M) root environments of a mature mesquite woodland in the Sonoran Desert of Southern California, and from variable depths (0–12 m) of non-phreatic mesquite ecosystems in the Chihuahuan Desert of New Mexico. They were tested for their ability to tolerate high salinity, and respire NO_3^- as mechanisms of free-living survival. Sixteen of 25 isolates were grown in yeast-extract mannitol (YEM) broth at NaCl concentrations of 2 (basal concentration), 100, 300, 500 and 600 mM, and their specific growth rates, cell dry weight and lag times were determined. Twenty of the 25 isolates were also grown in YEM broth under anaerobic conditions with or without 10 mM KNO_3. Three categories of NaCl salinity responses were observed: (1) eight isolates showed decreased specific growth rates at NaCl concentrations of 100, 300 and 500 mM, but they nevertheless remained viable at 500 mM NaCl concentration; (2) the specific growth rate of six isolates increased significantly at 100 and 300 mM NaCl; and (3) specific growth rates of two isolates were significantly greater than the base-rate at all concentrations of NaCl. Five of 11 of the *Bradyrhizobium* isolates tested respired NO_3^-, but showed no growth. Seven *Rhizobium* isolates, three from the deep (3.9–5 m) phreatic rhizobial community, and four from the surface community denitrified NO_3^- but only the isolates from the phreatic community displayed anaerobic growth. Long-term interactions between rhizobial and bradyrhizobial communities and the surface and phreatic root environments of the mature Sonoran Desert mesquite woodland appear to have selected for strains of NO_3^- respiring rhizobia, general salt tolerance of both rhizobial and bradyrhizobial symbionts, and strains of weak facultative halophilic bradyrhizobia. These survival characteristics of mesquite rhizobia may be important regarding mesquite's establishment and long-term productivity in marginal desert soils, and may provide novel types of rhizobia for food crops growing in harsh environments.
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Keywords: Mesquite; *Prosopis*; *Rhizobium*; *Bradyrhizobium*; Salt tolerance; Halophily; Nitrate respiration; Denitrification

1. Introduction

Mesquite (*Prosopis glandulosa* Torr) is a salt tolerant, phreatophytic N_2-fixing tree legume that is often the dominant woody legume in arid and semi-arid regions of the world (Burkhard and Simpson, 1977). In developing countries human need for fuel wood, food, fodder, erosion control and reclaiming salt-affected lands has brought attention to some species of *Prosopis* for their potential in agroforestry systems (Felker, 1979; Singh, 1995; Graham and Vance, 1999). Because establishment and primary productivity of mesquite in marginal soils may be improved when inoculated with selected rhizobia, an understanding of the ecology of *Prosopis* rhizobia, especially aspects that affect free-living survival and competition for sites of root nodulation, is important.

From a mesquite woodland near Harper's Well in the Sonoron Desert of Southern California, Jenkins et al. (1987) developed a collection of fast growing (FR) *Rhizobium* spp. and slow growing (SB) *Bradyrhizobium* spp. The isolates were recovered from the saline soil environments of the root system in the surface 0.4 m and from the phreatic root system contiguous to a permanent and stable groundwater source at 4–6 m depth. Based on historical evidence this woodland was established between 200 and 500 y ago after the recession of Lake Cahuilla (Weide, 1976). The dry subsoil between the two root systems isolated the two rhizobial communities from each other for several centuries (Jarrell and Virginia, 1990). Thus, each rhizobial community developed independent of one another in their respective soil environments.

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0038-0717/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.soilbio.2003.08.014

The rhizobial population from the permanently moist, relatively isothermal phreatic root system was dominated by *Bradyrhizobium* spp; whereas, the rhizobial community from the temperature and moisture fluctuating surface root system was comprised of both *Rhizobium* and *Bradyrhizobium* spp. (Jenkins et al., 1987). Further study of this collection of mesquite N_2-fixing rhizobia indicated that the *Rhizobium* spp. and *Bradyrhizobium* spp. from the two root systems comprised distinct phena (Waldon et al., 1989). In addition, based on restriction fragment length polymorphism of the *ndv* region of the chromosomes of selected isolates from the collection, Thomas et al. (1994) demonstrated that the two rhizobial populations had diverged. In contrast to findings of Hartel and Alexander (1984), and Marchall (1964) Waldon et al. (1989) observed that several *Rhizobium* isolates from the surface root environment grew better at 36 °C than at 26 °C. Their ability to tolerate the high temperatures often reached in surface soils in arid ecosystems (Wilkens, 1967) was, thus, selected as a survival mechanism.

Other survival mechanisms of importance for effective mesquite–rhizobia interactions are the ability to be salt tolerant, and, where the soil environment may become saturated or contain anoxic microsites, the ability to respire NO_3^-. Several species of *Prosopis* such as *P. glandulosa* Torr are salt tolerant and will form effective symbioses with rhizobial strains at salinities greater than those of sea water (Felker et al., 1981). Thus, salt tolerant strains of *Rhizobium* and *Bradyrhizobium* would be expected to exist that form N_2-fixing nodules on mesquite roots. Salt-tolerant strains of *Rhizobium* and *Bradyrhizobium* symbionts of tree legumes have been characterized (Hua et al., 1982; Yap and Lim, 1983; Bala et al., 1990). Likewise, one would expect *Rhizobium* and *Bradyrhizobium* isolates from the soil of the wet phreatic root system to respire NO_3^- because of the high probability of anoxic microsites. Denitrification and anaerobic growth resulting from nitrate respiration are wide-spread among *Bradyrhizobium* spp., but rare among *Rhizobium* spp. (Zablotowicz and Focht, 1978; Daniel et al., 1982; Van Berkum and Keyser, 1985). Studies of rhizobial salt tolerance and NO_3^- respiration on strains from various laboratory collections have given little attention to the ecology at their site of origin. Few have focused on a specific rhizobial population or community from a single host in a natural or agricultural ecosystem. Our objective was to assess the Na^+ tolerance and NO_3^- respiration capabilities of rhizobial and bradyrhizobial isolates from the surface and phreatic root systems of old mesquite trees from the woodland at Harper's Well.

2. Materials and methods

2.1. Rhizobial isolates tested

The rhizobial isolates tested are listed in Table 1. Methods of collection, locality of study sites, and characteristics of the soils and the rhizobial communities from which the isolates were obtained have been described (Jenkins et al., 1987, 1988; Virginia and Jarrell, 1983). Two *Rhizobium* and two *Bradyrhizobium* isolates from different mesquite ecosystems not high in soil salinity in the Chihuahuan Desert of New Mexico were included for comparative purposes (Jenkins et al., 1989). All of the isolates tested effectively nodulated mesquite seedlings (Jenkins et al., 1987, 1989).

Table 1
Mesquite-nodulating *Bradyrhizobium* and *Rhizobium* isolates examined in this study

	Site state	Soil depth (m)	Salinity (dS m^{-1})[a] or Extractable Na^+ (mM l^{-1})[b]
Bradyrhizobium isolates			
D7-5D	NM	5.0	0.4 dS m^{-1}
W2-.5D	NM	0.5	ND[c]
1J	CA	0.3	44.3 mM l^{-1}
10K	CA	0.4	100.3 mM l^{-1}
8E	CA	4.5	23.1 mM l^{-1}[d]
27D	CA	0.4	100.3 mM l^{-1}
43A	CA	5.0	23.1 mM l^{-1}
43B	CA	5.0	23.1 mM l^{-1}
43C	CA	5.0	23.1 mM l^{-1}
44A	CA	5.0	23.1 mM l^{-1}
44F	CA	5.9	23.1 mM l^{-1}
10E	CA	0.4	100.3 mM l^{-1}
10F	CA	0.4	100.3 mM l^{-1}
10H	CA	0.4	100.3 mM l^{-1}
Rhizobium isolates			
P1-11E	NM	11.0	0.2 dS m^{-1}
P2-1C	NM	1.0	0.3 dS m^{-1}
1E	CA	0.3	44.3 mM l^{-1}
10A	CA	0.4	100.3 mM l^{-1}
10D	CA	0.4	100.3 mM l^{-1}
HWT6P	CA	5.0	23.1 mM l^{-1}
8D	CA	4.5	23.1 mM l^{-1}
22A	CA	3.9	ND
27A	CA	0.4	100.3 mM l^{-1}
27C	CA	0.4	100.3 mM l^{-1}
27E	CA	0.4	100.3 mM l^{-1}

Isolates were from Na^+ rich surface and deep phreatic soil environments at the mesquite woodland at Harper's Well in the Sonoran Desert of Southern California (CA); four isolates were obtained from non-saline surface and deep soil (non-phreatic) environments from mesquite ecosystems in the Chihuahuan Desert of New Mexico (NM). The source of all isolates from California (with the exception of HWT6P for which the source is this paper) is Jenkins et al., 1987. The source of isolates from New Mexico is Jenkins et al., 1989.

[a] Jenkins et al., 1988.

[b] Virginia and Jarrell, 1983.

[c] [e]Not determined.

[d] Na^+ concentration of the ground water contiguous to the phreatic root system at 4.5–5 m depth (Virginia and Jarrell, 1983).

2.2. Halotolerance tests

Rhizobium and *Bradyrhizobium* isolates (Table 1) were taken from cultures stored in 20% glycerol at −20 °C,

and transferred to slants of yeast-extract mannitol (YEM) agar, pH 7 (Vincent, 1970). Transfers were made from the slants to YEM broth and inoculated flasks were incubated on a rotary shaker at 150 rev min^{-1} and 28 °C. At mid-exponential growth phase, 0.5 ml of broth culture was transferred to duplicate or quadruplicate 250 ml flasks of YEM (50 ml) broth with varying concentrations of NaCl above base concentration (2 mM): 100, 300, 500, and 600 mM. Isolates were grown with comparable concentrations of KCl. The inoculated flasks were held on a rotary shaker at 150 rev min^{-1} at 28 °C. Optical density was measured using a spectrophotometer (Bausch and Lomb Spectronic 21) at 660 nm (OD_{660}). Specific growth rates were calculated from the linear portion of the curve obtained by plotting the natural log of the OD_{660} readings against time. The end of the lag period was taken to be the time at which exponential growth began. Cell dry weights were determined by standard methods (Herbert et al., 1971): two 10 ml aliquots of a cell suspension were centrifuged at 1500*g* for 15 min; each pellet was washed once with 10 ml of sterile 150 mM NaCl, resuspended in 1 ml of 150 mM NaCl, and combined in a tared 2 ml microcentrifuge tube. The combined pellets were centrifuged at 11,000*g* for 5 min, the supernatant was discarded, the pellet dried at 100 °C, brought to room temperature in a desiccator, and weighed. Each determination of cell dry weight was made in duplicate on each of 16 cultures grown to the stationary phase. At the end of the growth rate experiments culture viability was determined by streaking a loopfull of culture onto YEM agar plates.

2.3. Nitrate respiration

The methods of Zablotowicz and Focht (1978) and Daniel et al. (1982) were adapted for determining NO_3^- respiration. Isolates were grown in YEM broth on a rotary shaker at 28 °C to late log phase. A loopfull of culture suspension was inoculated into sterile triplicate 16 by 100 mm screw-cap tubes containing 10 ml of sterile YEM broth amended with 10 mM KNO_3 and a Durham tube (Singleton and Sainsbury, 1987) for gas collection. As a control, triplicate tubes of YEM broth without KNO_3 were also inoculated as described above. Sterile liquid paraffin (0.5 ml) was layered over the liquid and then the tubes were capped. Inoculated tubes were incubated at 28 °C. Optical density (OD_{660}) of the cultures was measured at *d* 0, 2, 4, 6 and 19. Residual NO_3^- was measured by the salicylic acid method of Cataldo et al. (1975), and NO_2^- was measured by the method of Nicholas and Nason (1957).

2.4. Statistical analysis

Differences in specific growth rates, cell dry weight at each salt concentration, reduction in NO_3^- concentrations, and accumulation of NO_2^- were determined by one-way analysis of variance (ANOVA) and the means were separated either by Fisher's least significant difference (LSD) at $P < 0.05$, or by Dunnett's test at $P < 0.05$ (StatView for Macintosh, BrainPower, Calabasas, CA).

3. Results

3.1. Salt tolerance study

Results of the rhizobial growth studies (Table 2) indicated that the isolates tested can be grouped into three categories of NaCl-salt tolerance. Eight isolates in Category 1 were characterized by a decrease in specific growth rate with increased NaCl concentrations, although the specific growth rates of the *Rhizobium* isolates at the lowest concentration (2 mM NaCl) was maintained at 100 mM NaCl. The biomass accumulations as cell dry weight of these Category 1 isolates paralleled the specific growth rate patterns corresponding to each NaCl concentration (Table 3). The two *Bradyrhizobium* and two *Rhizobium* isolates from the Chihuahuan Desert site, where soil salinities were minimal (Jenkins et al., 1988), as well as a *Bradyrhizobium* and three *Rhizobium* isolates from the saline Sonoran Desert site in California (Jenkins et al., 1987; Virginia and Jarrell, 1983) fell into Category 1. All Category 1 isolates replicated (albeit slowly) and remained viable in 500 mM NaCl, and thus, can be considered salt tolerant.

The isolates in Category 2 had significantly increased specific growth rates in 100 mM NaCl, and, in some cases, in 300 mM NaCl. Unlike Category 1 isolates, several of these Category 2 isolates maintained a specific growth rate in 500 mM NaCl comparable to their rates at the basal NaCl concentration. Biomass accumulation as cell dry weight in 100 and 300 mM NaCl were comparable to or greater than growth in the basal NaCl concentration (Table 3).

The specific growth rates of the two *Bradyrhizobium* isolates in Category 3 were significantly accelerated by NaCl concentrations of 100, 300, 500, (Table 2) and even 600 mM (specific growth rate for 10E and 10F in 600 mM NaCl was 0.057 ± 0.007, and 0.041 ± 0.007 h^{-1}, respectively) compared to the basal medium. Because comparable concentrations of KCl inhibited replication of these isolates (data not shown), Na^+ appeared to be the factor stimulating growth. Chloride appeared not to be a stimulating growth factor as has been observed for some marine Pseudomonads (MacLeod and Onofrey, 1957). With the exception of growth in 100 mM NaCl, the increased biomass accumulation as cell dry weight in 300 and especially in 500 mM NaCl (Table 3) further substantiated the halophily of these isolates. Unlike Category 2 isolates, those in Category 3 showed a reduction in biomass accumulation as cell dry weight at a NaCl concentration of 100 mM compared to the standard YEM medium. This reduction at the intermediate NaCl concentration occurred despite increased specific growth rate, was repeatable, but cannot yet be explained.

Table 2
Mean specific growth rates ($\mu\ h^{-1}$) of mesquite-nodulating fast growing (FR) *Rhizobium* and slow growing (SB) *Bradyrhizobium* isolates at increasing concentrations of NaCl

Isolate	Genus[a]	Salt tolerance category[b]	[NaCl] (mM)				
			2	100	300	500	LSD[c]
10K	SB	1	0.066	0.041	0.047	0.019	0.021
D7-5D			0.034	0.019	0.16	0.007	0.003
W2-.5D			0.033	0.015	0.015	0.007	0.003
8D	FR		0.138	0.145	0.134	0.102	0.006
10A			0.106	0.104	0.068	0.034	0.002
10D			0.082	0.100	0.083	0.055	0.012
P1-11E			0.183	0.184	0.150	0.032	0.014
P2-1C			0.166	0.164	0.114	0.037	0.008
8E	SB	2	0.052	0.073	0.062	0.044	0.010
27D			0.020	0.042	0.033	0.023	0.001
43A			0.042	0.071	0.060	0.039	0.009
43B			0.034	0.076	0.062	0.034	0.001
44A			0.037	0.159	0.068	0.046	0.024
HWT6P	FR		0.131	0.153	0.140	0.112	0.004
10E	SB	3	0.033	0.065	0.103	0.093	0.007
10F			0.030	0.066	0.050	0.060	0.007

[a] SB, slow growing *Bradyrhizobium* isolates, and FR, fast growing *Rhizobium* isolates.
[b] Category 1 pertains to those isolates whose $\mu\ h^{-1}$ decreases with increased [NaCl]. Category 2 pertains to those isolates whose $\mu\ h^{-1}$ significantly increases at [NaCl] of 100 mM and then decreases with further increases in [NaCl] such that no significant difference exists between $\mu\ h^{-1}$ at the basal concentration of 2 mM and at 500 mM. Category 3 pertains to those isolates whose $\mu\ h^{-1}$ increases significantly at 100 mM NaCl and remains significantly greater than the basal concentration at 300 and 500 mM NaCl.
[c] Least significant difference at $P = 0.05$.

The duration of the lag phase for the category 3 isolates was significantly increased by NaCl concentrations of 500 mM (Table 4). The lag time of the Category 3 *Bradyrhizobium* isolates was further prolonged to 47 h by a NaCl concentration of 600 mM. All but one of the Category 2 isolates showed an increase in their lag phase at 300 and 500 mM NaCl. A similar pattern of a prolonged lag phase was observed in the four Category 1 isolates from the Sonoran Desert. The Category 1 isolates from the Chihuahuan Desert did not show a lag at any of the NaCl concentrations.

Table 3
Mean dry weight (μ g cell dry weight ml^{-1}) determinations of the isolates as affected by increasing NaCl concentration

Isolate	Genus	Salt tolerance category[a]	[NaCl] (mM)				
			2	100	300	500	LSD[b]
10K	SB	1	700	570	680	240	120
D7-5D			160	100	50	30	30
W2-.5D			200	100	60	30	30
8D	FR		820	810	710	440	40
10A			700	710	560	120	10
10D			630	700	660	360	140
P1-11E			480	600	360	20	40
P2-1C			560	570	410	90	30
8E	SB	2	330	350	290	150	30
27D			180	440	240	110	120
43A			380	430	380	160	30
43B			330	430	390	150	110
44A			350	310	420	240	20
HWT6P	FR		660	790	720	480	160
10E	SB	3	140	100	510	550	120
10F			390	200	240	410	110

[a] See explanation in Table 2.
[b] Least significant difference at $P < 0.05$.

Table 4
Mean lag time (h) of isolates as affected by increasing NaCl concentrations

Isolate	Genus	Salt tolerance category[a]	[NaCl] (mM)	
			300	500
			Lag time (h)	
10K	SB	1	14	14
D7-5D			0	0
W2-.5D			0	0
8D	FR		8	14
10A			14	28
10D			8	14
P1-11E			0	0
P2-1C			0	0
8E	SB	2	12	14
27D			23	48
43A			12	37
43B			10	36
44A			0	0
HWT6P	FR		5	10
10E	SB	3	0	12
10F			0	16

For NaCl concentrations of 2 and 100 mM no lag time was observed for any of the isolates; thus, lag times are given only for isolates grown in NaCl concentrations of 300 and 500 mM.
[a] See explanation in Table 2.

Table 5
Anaerobic NO_3^- reduction

	NO_3 remaining and NO_2 accumulated after incubation ($\mu g\ ml^{-1}$)	
	NO_3^-	NO_2^-
Rhizobium isolates		
HWT6 P[a]	8.17*	0.05
1E	636.23	0.21
8D	0.71*	0
10A	602.13*	0.26
10D	511.80*	0
22A	1.62*	0
27A	617.97	0.43
27C	541.33*	0.44*
27E	596.37*	0.18
Bradyrhizobium isolates		
1J	632.83	0.05
8E	661.67	0.09
10E	555.23*	0.09
10F	602.30*	0.10
10H	613.87	0.04
27D	612.83*	0.10
43A	571.70*	0.04
43B	616.77	0.08
43C	631.67	0.13
44A	624.33	0.04
44F	612.23*	0
NO_3 control	689.43	0.21

Mean reductions in NO_3^- concentration, accumulation of NO_2^- (means followed by * were significantly different from the NO_3^- control by Dunnett's test at $P < 0.05$), and gas production of fast growing (FR) *Rhizobium* and slow growing (SB) *Bradyrhizobium* isolates from the mesquite woodland at Harper's Well.

[a] *Rhizobium* isolate HWT6P was the only isolate to produce gas in Durham tubes.

3.2. Nitrate respiration

Three *Rhizobium* isolates, HWT6PE, 8D, and 22A from the phreatic rhizobial community denitrified, although only isolate, HWT6PE, produced gas (Table 5). Four other *Rhizobium* isolates, 10A, 10D, 27C and 27E, appeared to respire NO_3^- as indicated by the significant reduction in NO_3^-, but only isolate 27C accumulated a significant quantity of NO_2^-. Although five *Bradyrhizobium* isolates (four from the surface and one from the phreatic bradyrhizobial communities) reduced a significant amount of NO_3^-, they did not accumulate NO_2^-. The only isolates that showed growth with NO_3^- respiration were the three denitrifying *Rhizobium* isolates from the phreatic root zone (Fig. 1).

4. Discussion

4.1. Rhizobial isolates studied

The isolates tested in this study were representatives of wild-type communities from desert ecosystems where the host legume *P. glandulosa* is a dominant primary producer (Jenkins et al., 1987, 1988, 1989; Waldon et al., 1989). The specific growth rates of these isolates in YEM (with basal NaCl concentration) (Table 2) were within the ranges reported for *Bradyrhizobium* ($0.02–0.05\ h^{-1}$) by Stowers and Elkan (1984), and *Rhizobium* ($0.2 - 0.5\ h^{-1}$) by Hernandez and Focht (1984) of the cowpea group, the taxonomic group in which leguminous tree-nodulating rhizobia are customarily placed (Basak and Goyal, 1975; Allen and Allen, 1981). The only exception was isolate 10D whose specific growth rate was between fast and slow growing and was included with the *Rhizobium* isolates.

4.2. Salt tolerance categories

The Category 1 isolates contrast with those in other studies of rhizobial salt tolerance which showed cessation of growth at NaCl concentrations between 100 and 343 mM (Graham and Parker, 1964; Singleton et al., 1982; Zablotowicz and Focht, 1981). The *Rhizobium* isolates we examined in this study (category 1, Table 2) possessed growth patterns similar to the characterized salt tolerant strains of *Rhizobium japonicum* USDA 191 (Yelton et al., 1983), *Rhizobium* sp. UMKL 20 (Yap and Lim, 1983) and *Rhizobium* sp. from the tree legumes *Acacia*, *Leucena* and *Prosopis* (Bala et al., 1990).

All isolates in Category 2 were from the Sonoran Desert study site in California and originated from either the phreatic or the surface root environments (Table 1). Category 2 isolates appeared to exemplify adaptation to saline soils. This is not surprising because these *Bradyrhizobium* and *Rhizobium* communities have interacted with their host *Prosopis* for approximately 300 y (Waldon et al., 1989; Jarrell and Virginia, 1990). Pillai and Sen (1973) described six *Rhizobium* strains from *Dolichos lablab* that would fall into Category 2. Their growth rate in YEM was enhanced by 100 and 200 mM NaCl, but they did not survive in NaCl concentrations equal to, or greater than 500 mM.

The sustained stimulation of the specific growth rate of the two Category 3 *Bradyrhizobium* isolates at the higher NaCl concentrations is indicative of weak halophily (Singleton and Sainsbury, 1987), a novel physiological characteristic not previously observed in either *Rhizobium* spp. or *Bradyrhizobium* spp. Because the halophilic response was not obligate, it was, therefore, facultative.

The duration of the lag phase of growth for the two Category 3 isolates was in contrast to Gram-negative marine bacteria, whose optimal growth rates occurred between 200 and 500 mM NaCl (Berthelet and MacLeod, 1989). Since the growth experiments were initiated with inocula still in the exponential growth phase, the absence of a lag was expected for isolates growing in YEM at the basal NaCl concentration. The prolonged lag with increased NaCl concentration of Category 2 and 3 isolates may reflect a physiological response allowing them to continue

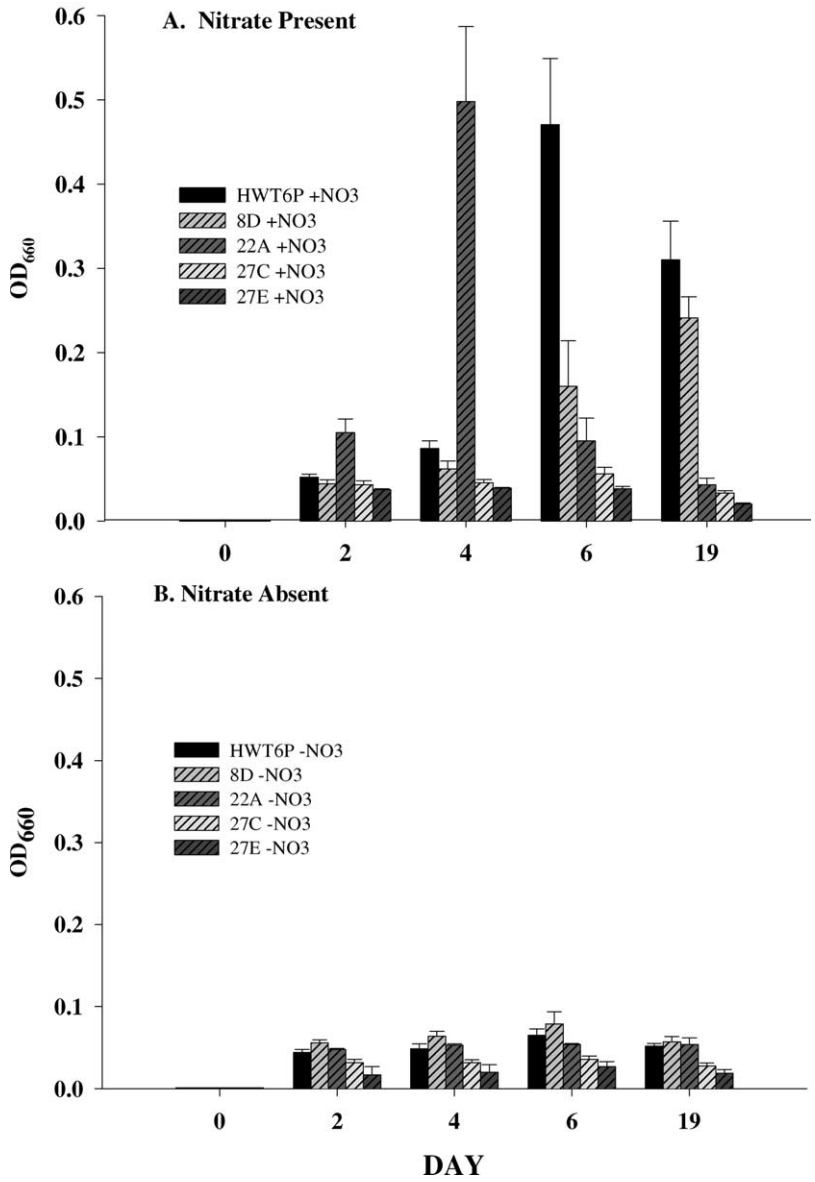

Fig. 1. Anaerobic growth as measured by duplicate optical density (OD_{660}) readings of five *Rhizobium* isolates, each in triplicate, in (A) the presence of NO_3^{-1}, and in (B) the absence of NO_3^{-1}.

replication at rates commensurate with (Category 2) or greater than (Category 3) rates associated with the basal NaCl concentration.

In developing effective rhizobial inocula for use in establishing legumes in saline soils, especially when the legume such as *Prosopis* is nodulated by both *Bradyrhizobium* and *Rhizobium*, knowing that one genus is generally more salt tolerant than the other may be helpful in inoculant development. Jenkins et al. (1989) reported a significant correlation between soil salinity and the distribution of *Prosopis*-nodulating *Rhizobium* and *Bradyrhizobium* in warm desert ecosystems. This correlation suggested that *Bradyrhizobium* populations would predominate over *Rhizobium* populations in saline soils. This latter report, a previous study (Singleton et al., 1982), and our data do not support the generalization that *Rhizobium* sp. are more salt-tolerant than *Bradyrhizobium* sp. (Bala et al., 1990; Graham and Parker, 1964).

4.3. NO_3 respiration

Of the 20 *Rhizobium* and *Bradyrhizobium* isolates tested for NO_3^- respiration, only the three *Rhizobium* isolates from the phreatic habitat at the mesquite woodland in California significantly reduced the concentration of NO_3^- and showed anaerobic growth. But only one of these three isolates showed gas production and as with the majority of the isolates tested, NO_2^- did not accumulate. Zablotowicz and Focht (1978) also observed several strains of *B. japonicum* and isolates of the cowpea miscellany group that appeared to denitrify without gas production. Without an accumulation of NO_2^-, however, either NO_3^- respiration was not

occurring by definition (Zablotowicz and Focht, 1978), or instead of accumulating NO_2^-, it was further reduced to NH_4^+ rather than N_2O (Halder and Chakrabartty, 1995). Rhizobial respiration of NO_3^- and denitrification resulting in anaerobic growth are wide-spread among *Bradyrhizobium* spp. and less frequent among *Rhizobium* spp. (Daniel et al., 1982; Van Berkum and Keyser, 1985). In contrast to this general observation, only one of the *Bradyrhizobium* isolates from the phreatic soil community denitrified; whereas, all three of the *Rhizobium* isolates from the phreatic soil community denitrified and showed anaerobic growth.

5. Conclusions

The *Rhizobium* and *Bradyrhizobium* isolates from the non-saline soil environments of Chihuahuan Desert site all fell into Category 1. In contrast, the *Rhizobium* and *Bradyrhizobium* isolates from the communities at the Sonoran Desert Site fell into all three salt-tolerance categories. The *Bradyrhizobium* isolates from the phreatic root environment, however, all fell into Category 2. The *Bradyrhizobium* isolates from the deep phreatic environment were shown to be physiologically distinct from the surface *Bradyrhizobium* isolates (Waldon et al., 1989) suggesting adaptation to their respective soil environments and diverging from the surface *Bradyrhizobium* community. Genetic analysis of phreatic and surface isolates also indicated that the two communities had diverged (Thomas et al., 1994). The presence of *Bradyrhizobium* in the surface soil environment in all three salt-tolerance categories suggests a gradation of adaptations to survival in the extremely fluctuating surface soil environment at the Sonoran Desert site (Jenkins et al., 1987) where soil solution salinity can range from -2.1 to -3.6 MPa (Virginia and Jarrell, 1983)—osmotic potentials comparable to 500–800 mM NaCl. Response of isolates to salinity in Category 1 suggested an incipient degree of salt tolerance. Category 2 isolates exhibited an apparent physiological response to intermediate NaCl concentrations (100–300 mM). The weak facultative halophilic response of the *Bradyrhizobium* isolates in Category 3 may reflect adaptation to the extreme fluctuations in resource availability which characterized the surface soil environment at the Sonoran Desert site (Jenkins et al., 1987). In the context of competition for root-nodule occupancy, the increased growth rate at the higher concentrations of soil salinity may enable the two Category 3 *Bradyrhizobium* representatives of the surface root community to compete with the *Rhizobium* present in this habitat. Over the centuries that the mesquite woodland at Harper's Well has existed the interactions between the trees' two separated root environments and the rhizobial and bradyrhizobial communities associated with them appeared to have selected for strains of NO_3^- respiring *Rhizobium*, general salt tolerance of both rhizobial and bradyrhizobial symbionts, and strains of weak facultative halophilic *Bradyrhizobium*. These survival characteristics of mesquite rhizobia may be important regarding mesquite's establishment and long-term productivity in marginal desert soils, and may provide novel types of rhizobia for food crops growing in harsh environments.

Acknowledgements

This study was supported by a grant from the Competitive Research Grant in Forestry from the US Department of Agriculture.

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